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The effect of microwave pasteurization on the composition of milk

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Abstract. Free amino acid and total amino acid content, water soluble vitamin content (vitamin C, B_1, B_2, B_6 and B_{12}) and utilizable lysine, lysinoalanine and hydroxymethyl furfurol content of milk were examined after the samples were pasteurized using different heat treatment.

They analyzed the effect of microwave treatment on the amino acids, free amino acid content and biological value compared to the conventional heat treatment technology. In was established that the two applied heat treatments caused practically no change in the amino acid composition of the milk protein neither in case of the essential nor in the case of the non-essential amino acids. The total free amino acid content of the raw milk (20.67 mg/100 g milk) reduced in the milk pasteurized in the traditional way to 8.02, whereas in the microwave pasteurized milk to 8.96 mg amino acid/100 g milk.

Key words and phrases: milk composition, mild pasteurization, microvawe pasteurization

They established, that after mild pasteurization, vitamin C content of milk hardly changed while during microwave pasteurization it decreased to less than its third value. Comparing the composition of raw milk with that of milk pasteurized using the two heat treatment methods, there was no important differences between the four vitamins B. Raw milk and milk pasteurized traditionally and by microwave contained hydroxymethyl furfurol not even in traces, and lysinoalanine content remained for all the three samples below the quantification limit. The utilisable lysine content of the raw milk and the two pasteurized milks was almost the same.

1 Introduction

Prior to the human consumption with the exception of some special dairy products milk has to be pasteurized in order to kill the pathogenic microbes. Pasteurization should be carried out by minimizing the effect on the composition and organoleptic properties of the raw milk. Beside the conventional pasteurization procedures recently the microwave treatment has been used.

In the food industrial practice one of the application fields of the microwave technique is the enhancement of the microbiological safety of the products by pasteurization. According to Pozar [8], during the microwave processes mainly the frequency of 2450 MHz, and in some cases the frequency of 915 MHz have been used. The microwave pasteurization is a promising method as based on the experiences the foodstuffs are damaged less than during the conventional heat treatment, due to the short treatment and radiation time [4, 9, 10, 11]. Sieber et al. [9] when treated milk with microwaves could not detect health-damaging effects. Özilgen and Özilgen [7] examined the kinetics of killing of *Escherichia Coli* by heat during microwave pasteurization, and found that the microwave treatment was applicable at lower temperatures both for pasteurization and sterilization. One of the greatest advantages of the method over the conventional heat treatment is that the product can be treated also when it is already in a closed packaging and by this the product can be stored for a significantly longer time even without added preservatives.

The aim of our research was to investigate if there is a difference between the conventional and microwave pasteurization regarding their effect on the composition of milk.

2 Material and methods

2.1 Milk samples examined

The examined raw milk was obtained from a dairy company in Hargita county, Romania. The normal (mildly) pasteurized milk was obtained by a heat treatment at 72 °C for 40 sec. In case of microwave pasteurized milk samples the milk was pre-heated to 63 °C in a plate heat-exchanger, and was heated to 68 °C by treatment with microwave of 2.45 GHz frequency and was kept at this temperature for 40 sec. The experimental pasteurization equipment consisted of three ALASCA household microwave ovens that were connected into cascade with the ovens placed above each other. Inside of each oven a glass spiral was placed horizontally, that exited the instrument on the rear side. The spiral had an inner diameter of 18 cm, the glass tube had an ID of 20 mm. The three spiral tubes were connected into cascade by flexible tubes. The microwave pasteurization equipment had a capacity of 200 dm^3/h. The raw milk had a fat content of 3.65%, fat free dry matter content of 8.77%, density of 1.028 g/dm^3, total CFU of 2,600,000/cm^3, and *E. coli* number of 1,000/cm^3. After microwave pasteurization at 68 °C total CFU reduced to 1,200/cm^3, no *E. coli* was found, while all the other parameters remained unchanged. The experiments were repeated three times, and the three milk samples from each experiment were analysed.

2.2 Pretreatment and analysis of the samples

Pretreatment of the samples. After sampling the milk samples were cooled down to –25 °C immediately. Sample preparation and analysis were carried out at the Department of Food Science of the Sapientia Hungarian University of Science of Transylvania, Csíkszereda Campus.

The samples were centrifuged at 8,000 g for 15 min in order to remove the cellular elements, then the milk was defatted. Subsequently, to 5 cm^3 of the sample 5 cm^3 of 50% trichloroacetic acid was added and left standing for 20 min. The precipitate was centrifuged for 10 min at 10,000 g [1]. The supernatant was separated, and its vitamin, free amino acid and free D-amino acid content were determined.

Determination of the amino acid composition of milk samples. The total amino acid content was determined after protein hydrolysis [5]. The amino acid analysis was carried out using an INGOS AAA400 amino acid analyzer, on an Ostion Lg ANB cation-exchange resin (35 cm×0.37 cm sized

glass column). Absorbance of the ninhydrin derivatives of the amino acids was measured at the wavelengths of 440 and 570 nm.

During the determination of the free amino acid and free D-amino acid content, derivatization and the analysis were performed using a Varian Pro Star HPLC apparatus. For data collection and evaluation Varian Star 6.0 software was used.

From the free amino acids cyclic derivatives were formed with o-phthaldialdehyde (OPA) and 2-mercaptoethanol [2]. Separation was carried out on a 150×4 mm Supelcosil-C18 column using a two-component (methanol and sodium acetate buffer) gradient system.

From the amino acid enantiomers diastereomer pairs were formed with o-phthaldialdehyde (OPA) and 1-thio-β-D-glucose tetraacetate (TATG) [3]. The separation was carried out on a 125×4 mm Superspher-C8 analytical column using a three-component (methanol, phosphate buffer and acetonitrile) gradient system.

Flow rate was 1 cm^3/min in both cases. The derivatives were detected using a fluorescent detector (excitation wavelength: 325 nm, emission wavelength: 420 nm).

Determination of the vitamin content of milk samples. Separation of vitamins C and B was carried out by reversed-phase HPLC on a Supercosil (C18) column (150×4 mm id). The HPLC system consisted of 2 Varian ProStar 210 pumps, a Varian ProStar 320 UV detector and a Varian ProStar 363 fluorescent detector. Flow rate was 0.8 cm^3/min.

Composition of the mobile phase: For determination of vitamins B: 50:50% mixture of methanol and phosphate buffer. For determination of vitamin C: 10:90% mixture of acetonitrile and acetic acid (0.4%).

Isocratic analysis was performed with a 20 µl injection volume and detectation at 254 nm. Before injection samples were filtrated on a 0.20 µm Millipore (Millipore, Milford, MA, USA) filter. The results were recorded and evaluated with a Varian ProStar 6.0. software.

HMF was also determined by HPLC, using the Varian Pro Star HPLC apparatus, on a Supelcosil C18 column (150×4.6 mm id) and the Pro Star 320 UV-VIS detector. A two-component eluent consisting of acetonitrile and water (5:95, v/v) was used. Flow rate was 1 cm^3/min. HMF was detected at 284 nm.

Utilizable lysine content was determined after derivatization with 2,4-dinitro-1-fluorobenzene (DNFB). Dinitrophenyl-ϵ -amino-lysine (DNP lysine) was determined using amino acid analyzer (INGOS AAA). Lysinoalanine was deter-

mined by the INGOS AAA amino acid analyzer, under conditions similar to those of the normal amino acid analysis. Lysinoalanine elutes after tyrosine and phenylalanine, and before the basic amino acids.

3 Results

3.1 Total amino acid content of milk samples

Amino acid content of the raw milk, the conventionally pasteurized milk (normal milk) and microwave pasteurized milk (MW milk) is shown in *Table 1.*

Table 1: Amino acid content of milk samples heat treated by different manner (n=5)

Amino acid	**Milk samples**					
	Raw milk		**Milk pasteurized**			
			by mild heat treatment		**by microwave heat treatment**	
	g AA/ 100 g milk	g AA/ 100 g protein	g AA/ 100 g milk	g AA/ 100 g protein	g AA/ 100 g milk	g AA/ 100 g protein
Asp	0.216	6.8±0.28	0.207	6.9±0.35	0.212	6.8±0.37
Thr	0.124	3.9±0.32	0.118	3.9±0.29	0.123	4.0±0.36
Ser	0.165	5.2±0.26	0.158	5.3±0.25	0.159	5.1±0.30
Glu	0.694	21.8±0.22	0.650	21.6±0.28	0.669	21. 6±0.24
Pro	0.376	11.8±0.31	0.343	11.4±0.36	0.356	11.5±0.33
Gly	0.058	1.8±0.29	0.055	1.8±0.32	0.058	1.9±0.26
Ala	0.101	3.2±0.36	0.098	3.3±0.31	0.100	3.2±0.34
Cys	0.021	0.7±0.20	0.022	0.7±0.27	0.023	0.7±0.24
Val	0.185	5.8±0.25	0.173	5.8±0.32	0.180	5.8±0.28
Met	0.097	3.0±0.30	0.090	3.0±0.21	0.090	2.9±0.26
Ile	0.154	4.8±0.23	0.146	4.9±0.33	0.140	4.5±0.28
Leu	0.284	8.9±0.28	0.265	9.0±0.24	0.276	8.9±0.31
Tyr	0.130	4.1±0.19	0.127	4.2±0.25	0.132	4.3±0.26
Phe	0.140	4.4±0.22	0.135	4.5±0.28	0.139	4.5±0.31
His	0.086	2.7±0.25	0.079	2.6±0.21	0.080	2.6±0.25
Lys	0.232	7.3±0.30	0.223	7.4±0.24	0.236	7.6±0.28
Arg	0.073	2.3±0.31	0.070	2.3±0.25	0.078	2.5±0.22

After a statistical analysis of the results no significant difference could be established between the amino acid content of the raw milk and the milk pasteurized in the two manners ($P \leq 0.05$). Thus, it can be concluded that

the two kinds of heat treatment practically did not cause any change in the amino acid content of the milk regarding the essential and non-essential amino acids. Change in the ammonia content was also minimal: it was 0.047% for the conventionally pasteurized milk and 0.048% for the microwave pasteurized milk, which was practically identical with the ammonia content of the control.

In *Table 1* the results can be seen also in g amino acid/100 g protein unit which shows the proportion of the individual amino acids in percentage of the milk protein and gives information on the quality of the milk protein. As the amino acid composition expressed in g amino acid/100 g sample hardly changed due to the different treatments, and the total amount of amino acids was nearly the crude protein content for all the three samples, therefore no difference was found in the amino acid composition of the protein between the three milk samples. It can be concluded that the heat treatment we applied did not affect the amino acid content of the milk (g amino acid/100 g sample), and did not influence the amino acid composition of the protein (g amino acid/100 g protein) and the biological value of the protein. Calculating the biological value of milk protein according to Morup and Olesen [6] 81.2 was obtained for the control milk sample, 80.9 for the conventionally pasteurized milk, while 80.8 for the microwave pasteurized milk. These results show that the heat treatment we applied had no effect at all on the biological value of the milk protein.

3.2 Free and free D-amino acid content of milk samples

Table 2 shows the free amino acid content of raw milk and the pasteurized milks in mg amino acid/100 g milk, as well as percentage of free amino acids.

Looking at the free amino acids different conclusions are obtained than in case of the total amino acid content. Total free amino acid content of the raw milk was measured to be 20.67 mg/100 g milk. This value reduced to 8.02 mg amino acid/100 g milk for the traditionally pasteurized milk and to 8.96 mg amino acid/100 g milk for the microwave pasteurized milk, respectively. Among the amino acids the amount of phenylalanine, histidine, leucine, lysine, methionine, valine, aspartic acid, glutamic acid, proline and tyrosine decreased substantially and these differences could be also confirmed statistically. Though some differences were detected in case of the means of isoleucine, threonine, alanine, arginine, cystine, glycine and serine these differences did not proved to be significant.

The amount of phenylalanine, histidine, leucine, lysine, methionine, valine, aspartic acid, proline and tyrosine decreased considerably, while that

of isoleucine, threonine, alanine, arginine, cystine decreased to a less extent, while some increase was obtained for glycine and serine.

Table 2: Free amino acid content of differently treated milk samples (n=5)

Amino acid	Milk samples					
	Raw milk		Milk pasteurized			
			by mild heat treatment		by microwave heat treatment	
	mg AA/ 100 g milk	%	mg AA/ 100 g milk	%	mg AA/ 100 g milk	%
Asp	$1.66^{a}\pm0.324$	8.0	$0.41^{b}\pm0.182$	5.1	$0.46^{b}\pm0.277$	5.1
Thr	$0.14^{a}\pm0.113$	0.7	$0.09^{a}\pm0.125$	1.1	$0.07^{a}\pm0.110$	0.8
Ser	$0.07^{a}\pm0.042$	0.3	$0.16^{a}\pm0.037$	2.0	$0.08^{a}\pm0.025$	0.9
Glu	$7.07^{a}\pm0.456$	34.2	$4.75^{b}\pm0.315$	59.2	$5.15^{b}\pm0.422$	57.4
Pro	$4.23^{a}\pm0.337$	20.5	$0.14^{b}\pm0.091$	1.7	$0.23^{b}\pm0.113$	2.6
Gly	$0.33^{a}\pm0.234$	1.6	$0.74^{a}\pm0.421$	9.2	$1.01^{a}\pm0.248$	11.3
Ala	$0.50^{a}\pm0.200$	2.4	$0.28^{a}\pm0.112$	3.5	$0.37^{a}\pm0.154$	4.1
Cys	$0.06^{a}\pm0.032$	0.3	$0.01^{a}\pm0.014$	0.1	$0.02^{a}\pm0.018$	0.2
Val	$1.04^{a}\pm0.066$	5.0	$0.14^{b}\pm0.009$	1.7	$0.18^{b}\pm0.011$	2.0
Met	$0.27^{a}\pm0.098$	1.3	$0.01^{b}\pm0.008$	0.1	$0.03^{b}\pm0.016$	0.3
Ile	$0.14^{a}\pm0.075$	0.7	$0.05^{a}\pm0.030$	0.6	$0.04^{a}\pm0.024$	0.4
Leu	$0.54^{a}\pm0.188$	2.6	$0.04^{b}\pm0.022$	0.5	$0.06^{b}\pm0.019$	0.7
Tyr	$1.40^{a}\pm0.323$	6.8	$0.07^{b}\pm0.034$	0.9	$0.08^{b}\pm0.038$	0.9
Phe	$1.03^{a}\pm0.218$	5.0	$0.08^{b}\pm0.028$	1.0	$0.08^{b}\pm0.021$	0.9
His	$0.45^{a}\pm0.107$	2.2	$0.14^{b}\pm0.083$	1.7	$0.17^{b}\pm0.075$	1.9
Lys	$0.76^{a}\pm0.026$	3.7	$0.20^{b}\pm0.012$	2.5	$0.20^{b}\pm0.017$	2.2
Arg	$0.10^{a}\pm0.051$	0.5	$0.10^{a}\pm0.042$	1.2	$0.10^{a}\pm0.063$	1.1

$^{a\,b}$Averages in one row with common superscript do not differ ($P\leq0.05$)

In case of the non-essential amino acids the biggest decrease was experienced for proline and aspartic acid, at the same time in case of glutamic acid which is present in the highest amount, the change is relatively negligible. Summarized, it can be said that the essential amino acid content of the raw milk with the exception of arginine decreases considerably, and also the non-essential amino acids – except glycine and serine – decrease due to the heat treatment.

As the raw milk sample was immediately frozen down along with the heat-treated samples, it could be excluded that the higher free amino acid content was due to the souring of the raw milk, the growth of lactobacilli. It can be assumed that this huge decrease in the amount of free amino acids is due to the technological intervention only. There are two possibilities regarding the changes occuring during the heat treatment. As free amino acids are significantly more reactive than those bound in the peptide chain, it is possible that they reacted with milk sugar during the heat treatment resulting in Maillard reaction products. It is also supported by the fact that there was a decrease of 4 to 5% in the utilizable lysine content due to the heat treatment. This minimal decrease can be a result of the transformation of the free lysine and not of lysine bound in protein. For the rest of the amino acids there is no experimental evidence supporting this theory.

The other possibility may be that the whey proteins coagulated during the heat treatment could absorb the free amino acids on their surface so strongly that we could not remove them from the surface in the course of the determination. In fact, this latter possibility would be the most useful for the practice since no free amino acids would remain in the whey but they would enhance the biological value of the dairy product by being bound on the surface of the protein.

In the course of the examination of the free D-amino acids we could detect D-aspartic acid, D-glutamic acid and D-alanine in the milk samples. Amino acids other than these ones could not be detected at the sensitivity level of our HPLC system. The amount of D-amino acids practically did not change due to the different heat treatments. The amount of D-Asp varied from 0.016 mg/100 g milk in milk pasteurized conventionally to 0.017 mg/100 g milk, to 0.018 mg/100 g milk in the microwave pateurized milk, D-Glu changed from 0.053 mg/100 g milk to 0.052 and 0.054 mg/100 g milk, whereas D-Ala from 0.043 mg/100 g to 0.049 and 0.046 mg/100 g milk. Thus, it can be concluded that during the pasteurization with the applied temperature and time combinations did not change the D-amino acid content of the raw milk. In this respect between the two heat treatment procedures cannot be distinguished.

3.3 Vitamin B and C content of milk

We chose these vitamins because both vitamin C and vitamins B are very sensitive to the technological interventions, especielly on heat treatment. Ascorbic acid and vitamin B content of the raw milk and the differently heat treated milk are shown in the *Table 3.*

As it can be seen vitamin C content hardly changed due to mild pasteurization, however, it reduced to less than its one-third. This is very surprising because the microwave pasteurization was carried out at a lower temperature (68 °C) than the conventional (72 °C) therefore it appears that in the microwave pasteurization not only the temperature but also the energy of the microwave could play a role in the deterioration of the vitamin C content.

Table 3: Vitamin C and B content of raw milk, and milk samples after mild and microwave pasteurization (n=5)

Vitamin contenet mg/dm^3	**Milk samples**		
	Raw milk	**Milk pasteurized by milh heat treatment**	**Milk pasteurized by microwave**
Vitamin C	$22.71^{a}\pm1.273$	$22.11^{a}\pm1.106$	$6.25^{b}\pm0.825$
Vitamin B_1	$0.39^{a}\pm0.083$	$0.27^{a}\pm0.075$	$0.26^{a}\pm0.062$
Vitamin B_2	$1.81^{a}\pm0.324$	$1.63^{a}\pm0.193$	$1.65^{a}\pm0.215$
Vitamin B_6	$0.52^{a}\pm0.153$	$0.48^{a}\pm0.172$	$0.46^{a}\pm0.144$
Vitamin B_{12}	$0.004^{a}\pm0.0021$	$0.004^{a}\pm0.0014$	$0.003^{a}\pm0.0012$

^{ab} Averages in one row with common superscript do not differ p≤0,05.

Out of vitamins B we measured, vitamin B_1 is the most heat sensitive, while the other three have a higher resistance against heat impact. Accordingly, vitamin B_1 content decreased by approx. 33.3% due to both pasteurization procedures. In case of vitamins B_2, B_6 and B_{12} this decrease was around 10-11%, however, it did not prove to be a significant difference.

3.4 Hydroxymethyl furfurol content of milk

The quality of conventionally and microwave pasteurized milk is determined by measuring the hydroxymethyl furfurol (HMF) content. HMF is always present when foods with high protein and sugar content are heat-treated. *Table 4* shows HMF content of the differently heat treated milk samples, sweetened condensed milk and milk powder in μg HMF/100 g sample.

Based upon the results it can be said that the raw milk, the conventionally pasteurized and microwave pasteurized milk did not contain HMF even in traces, thus, in this respect the two pasteurization procedures are equal. In

Table 4: HMF (hydroxymethyl furfurol) content of milk sample with different heat treatment, sweetened condensed milk and milk powder (μg HMF/100 g sample)

Sample		HMF content μg HMF/100 g sample
Raw milk		-
Milk pasteurized by mild heat treatment		-
Milk pasteurized by microwave		-
Sweetened condensed milk	Mean	127
Milk powder	Mean	684

order to check the suitability of the analytical method, we determined the HMF content of a commercially obtainable sweetened condensed milk and a milk powder in three repetitions.

It was established that the sweetened condensed milk contained on the average 127 μg HMF/100 g, while the milk powder contained on the average 684 μg HMF/100 g sample. Comparing these values, it was found that the milk powder contains more HMF than the condensed milk. The reason for this is that milk powder is produced at a higher temperature than the condensed milk, and the formation of the products of the Maillard reaction accelerates at a higher temperature. In the milk powder 600–700 μg HMF/100 g was found.

3.5 Utilizable lysine and lysinoalanine content of milk

Parallelly with the analysis of the HMF content of the milk samples we examined the utilizable lysine and the lysinoalanine content of the milk samples heat-treated differently. The results are shown in *Table 5.*

No lysinoalanine could be detected above the level of the sensitivity of the measurement either in the raw milk or the two heat-treated milk samples. This means that neither threonine (perhaps serine) that is very sensitive to heat treatment, nor cysteine and cystine that are sensitive to heat treatment and oxidation, decomposed considerably, as these two amino acids are the main precursors of lysinoalanine.

Utilizable lysine content of the raw milk was measured to be 0.229%, that of the conventionally pasteurized milk to be 0.217%, whereas that of microwave pasteurized milk to be 0.219%, this is not a significant difference ($P \leq 0.05$).

Table 5: Utilisable lysine and lysinoalanine content of milk samples with different heat treatment (n=5)

Component examined	Milk sample		
	Raw milk	**Milk pasteurized by mild heat treatment**	**Milk pasteurized by microwave**
Utilisable lysine content, %	0.229±0.075	0.217±0.093	0.219±0.104
Lysinoalanine content, mg/dm^3	<5	<5	<5

It can be concluded that during the heat treatment we applied the ϵ-amino group of lysine which is very sensitive to the reducing sugars and heat did not convert to such an extent that could influence its biological utilizability. The around 4–5% difference in the utilizable lysine content indicates that some Maillard reaction product formed.

From our examinations it can be concluded that the two heat treatment methods can be considered as equal in this respect, and neither of them decreased substantially the utilizability of lysine, one of the most important essential amino acids, and neither of them resulted in a considerable lysinoalanine content.

4 Summary

In the course of the experiment we examined milk samples pasteurized using different heat treatment procedures at a dairy company in Hargita County, Romania. We analyzed the effect of the microwave treatment on the components, and compared the data with those obtained in case of the traditional heat treatment.

The two heat treatments applied practically did not cause any change in the amino acid composition of the milk protein regarding. For the free amino acids a considerable difference was obtained between the raw milk and the milk samples heat-treated differently, but between the two heat treatment methods cannot be distinguished in the respect of free amino acids.

Due to mild pasteurization vitamin C content of the milk hardly changed,

while during the microwave pasteurization it reduced to less than its one-third, compared to the raw milk. In case of vitamin B_1 it was established that a loss of 30–40% can be expected, while for the other three vitamins B this decrease was only around 10%.

During the examinations we could not detect hydroxymethyl furfurol and lysinoalanine in any of the milk samples, and we could not establish a significant decrease in the utilizable lysine content due to the pasteurization procedures.

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